



Case report

Lack of impairment due to confirmed codeine use prior to a motor vehicle accident: Role of pharmacogenomics

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ABSTRACT

Background: We examined forensic serum toxicology and pharmacogenomics data from a woman on codeine shortly before she caused a motor vehicle accident.

Methods: A woman driving erratically collided with a parked car of a highway seriously injuring 2 men working to repair the parked vehicle. The woman tested positive for codeine, acetaminophen and barbitol. She had been taking these medications for 20 years due to migraine headache. Serum toxicology and genotype analysis for cytochrome P450, UDP glucuronosyltransferase, and other metabolizing enzymes were measured.

Results: The woman was tried and convicted of driving under the influence resulting in bodily harm and was sentenced to 6 years. Toxicology results on peripheral blood showed a total and free codeine of 840 and 348 µg/L, respectively, and total morphine of 20 µg/L (17, 3, and 0 µg/L for morphine-3-glucuronide, morphine-6-glucuronide, and free morphine, respectively). She was heterozygous for CYP 2D6 *2/*4 (extensive/poor metabolism) and heterozygous for UGT 2B7 *1/*2 (extensive/ultra-rapid metabolism). The woman was also taking fluoxetine and bupropion which are strong inhibitors of CYP 2D6.

Conclusions: Based on her genotype and phenotype and reports by the arresting officer, we suggest that the subject in question was not intoxicated by opiates at the time of her motor vehicle accident and may have been falsely incarcerated.

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1. Introduction

Codeine is a widely for pain management and cough suppressant. It is available as a prescription drug in combination with other analgesics such as acetaminophen.¹ The majority of administered codeine (75–90%) is metabolized to codeine-6-glucuronide (C6G), while a trace amount is metabolized to norcodeine, or excreted directly into urine (about 5% each).² Between 0 and 15% of codeine is metabolized to morphine by cytochrome (CYP) 2D6. The variability is due to genetic variances in 2D6. The majority of individuals are extensive metabolizers (EM) and have the *1/*1 genotypes. Individuals with the CYP 2D6 gene duplication are ultra-rapid metabolizers (UM) and produce excess amounts of morphine from codeine.³ In contrast, individuals who are poor metabolizers (PM) for 2D6, e.g., *4/*4, are unable to produce morphine from codeine. Intermediate metabolizers (IM), e.g., *10/*10, or those

heterozygous for EM and PM have enzyme metabolic rates in between these two phenotypes.

The glucuronation of codeine to C6G is catalyzed by uridine diphosphate glucuronyltransferase (UGT) 2B7, while 5% of codeine is metabolized into norcodeine by CYP 3A4.³ Morphine is also glucuronidated by UGT 2B7 to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Variances in UGT 2B7 and UGT 1A1 can also affect the level of glucuronidation.⁴ Individuals with the *2 variant are UM and produce more glucuronide metabolites (C6G, M3G and M6G). While there are genetic variances in CYP 3A4, the enzyme responsible for producing norcodeine, however, because neither codeine nor norcodeine are strongly active, these mutations have no impact.

While morphine and the M6G metabolite are acknowledged as pharmacologically active, there is debate regarding the analgesic effect of codeine and the C6G metabolite. The pharmacologic effects of opiates are determined by its affinity to the µ receptor within the brain and central nervous system. Studies in rat brain homogenates have shown that inhibition constant of morphine and M6G are some about 30 fold higher than for M3G, and 200-fold higher for codeine and C6G.⁵ In a study of individuals undergoing

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hysterectomy, codeine was sufficient to relieve postoperative pain in 9 of 10 individuals who were CYP 2D6 EM (codeine dosage 12.6 mg/h).⁶ There was no relief of pain in the 1 individual who was a 2D6 PM despite a high codeine dose. In another study, genotyping was conducted in oral opiate dependent subjects.⁷ Poor metabolizers were not seen in opiate dependent patients. As a control, the incidence of PMs among individuals who were not drug dependent compared to individuals who were dependent to drugs not metabolized by 2D6 was 6.5% and 4%, respectively. In contrast, individuals who are 2D6 UM develop morphine toxicity due to higher than average conversion from codeine to morphine. Collectively, these data suggest that the major pharmacologic effect of codeine is through morphine and morphine metabolites, particularly M6G. Neither codeine nor C6G have any significant pharmacologic activity.

2. Case report

A witness observed the female defendant as driving erratically on an interstate highway at 50–60 miles per hour before colliding with a vehicle parked along the highway. The woman's minivan struck and seriously injured two men who were working to repair their disabled vehicle while on the shoulder. The defendant disclosed to the highway patrolman that she did not drink any alcohol, but had taken fluoxetine, bupropion, nortriptyline, valproic acid, trazodone, and over-the-counter medications a few hours before the accident. The defendant had a 20-year history of codeine, acetaminophen and butalbital for relief of migraine headaches. The antidepressant medications were prescribed by her clinical psychiatrist because of depression diagnosed 4 years earlier. In addition, she had been prescribed promethazine with codeine (10–20 mg) syrup every 4 h. A licensed paramedical technician performed an assessment of impairment of the defendant by drugs. The medic found the defendant to be alert, oriented x4, no absence or altered level of consciousness, back pain, dizziness, nausea, vomiting chest pain or shortness of breath. Her blood pressure and pulse rate was regular. Her pupils were equal and reactive to light. She displayed strong, equal movement in her arms and legs. Using the Glasgow Coma Scale, the defendant scored the highest marks for eyes, verbal and motor function. The paramedic concluded that the defendant did not note anything that would lead him to suspect that the woman was under the influence of a narcotic analgesic. Peripheral blood was collected from the defendant about 2 h after the accident. The results are shown in Table 1. Based on the blood toxicology results, the defendant was tried and convicted of driving under the influence resulting in bodily and sentenced to 6 years imprisonment.

Table 1
Blood toxicology and pharmacogenomics results for the case report.

General drugs	Concentration	Opiates	Concentration
Acetaminophen	1900 µg/L	Butalbital	400 µg/L
Lamotrigine	760 µg/L	Trazodone	80 µg/L
Valproic acid	1200 µg/L	Alcohol	0 mg/dL
Free codeine	348 µg/L	Total codeine	840 µg/L
Free morphine	0 µg/L	Total morphine	20 µg/L
Morp.-6-glucuronide	3 µg/L	Morp.-3-glucuronide	17
Genotypes			
CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2E1, 3A4	Wildtype		
UDP glucuronosyltransferase 1A1	Wildtype		
CYP 2C19	*1/*17 (normal/increased)		
CYP 2D6	*2/*4 (normal/none)		
CYP 3A5	*3C/*3C (none/none)		
UDP glucuronosyltransferase 2B7	*1/*2 (normal/increased)		
N-acetyltransferase 1 and 2	*4/*4 (rapid/rapid acetylator)		

3. Methods

Free codeine, morphine, and morphine glucuronides (M3G and M6G) were measured from the Santa Clara County Crime Laboratory using liquid chromatography-tandem mass spectrometry without hydrolysis. Total codeine and total morphine were measured at the Central Valley Toxicology Laboratory using gas chromatography-mass spectrometry following hydrolysis of glucuronides. The total morphine result between the laboratories was in agreement with each other. Blood was obtained from the defendant while in prison, the DNA extracted using QIAamp® (Qiagen), amplified by polymerase chain reaction, and tested for pharmacogenetic variances. Genotyping for 14 relevant pharmacogenomic relevant targets was conducted using Sequenom Inc., San Diego, CA. As this was a single case report, oversight by the University of California Committee on Human Health was deemed unnecessary, nevertheless, consent by the subject was granted for the release of medical information and for the pharmacogenomics analyses.

4. Results

The toxicology test results are summarized in Table 1. The free codeine concentration was considerably higher than what is typically seen after a single 30 mg dosing where results for a PM and EM were <100 µg/L.⁸ However, these results are similar values seen in a clinical scenario of chronic pain management were reported doses were as much as 10-fold higher.⁹ Table 1 also lists the pharmacogenetic results. Of particular note was that the subject was heterozygous for CYP 2D6 *2/*4 (wildtype and PM), heterozygous for UGT 2B7 *1/*2 (wildtype and UM), and homozygous for CYP3A4 *1/*1 (wildtype). This indicates that the subject had reduced ability than normal individuals to metabolize codeine to morphine, and increased ability than normal to metabolize what morphine that is formed from codeine to M3G and M6G. The conversion of codeine to C6G is also accelerated but C6G blood levels were not available. The absence of free morphine in the defendant's blood is evidence of this atypical rate of conversion.

Table 2 compares blood opiate test results from this case report to data from a woman who was a PM and 2 women who were EMs. The total morphine to total codeine ratio in a PM subject was considerably lower than the 2 cases of EMs (1.5% vs. 9.0% and 7.1%, respectively). In a study of 34 cases of codeine-related deaths, Frost et al. performed genotype and quantitative opiate analysis for codeine, morphine and morphine glucuronides.¹⁰ The mean and median 5.7 and 3 µg/L ($n = 18$) for EMs ($n = 18$) genotypes which was higher than 4.3 and 1.0 µg/L for IM ($n = 12$). Although the range of ratios in the Frost report was broad making it difficult to make any definitive conclusions, the defendant in this case report had a

Table 2
A comparison of blood opiate concentrations to literature report.^a

Opiate	Poor metabolizer	Extensive metabolizer ^b	Extensive metabolizer ^b	This case
Free codeine	617	407	578	348
Total codeine	2119	1206	2585	840
Free morphine	5.3	47.5	29.5	0.0
Morphine-3-glucuronide	22	50.5	134	17
Morphine-6-glucuronide	4.7	11	21	3
Total morphine	32	109	184	20
Total morphine/ total codeine, %	1.5	9.0	7.1	2.4
Free morphine/ morphine gluc. %	20.0	77.0	19.0	0.0

^a Data taken from reference#. ^b Results in µg/L.

^b Representative cases chosen because opiate results were similar to the case report.

codeine-to-morphine ratio of 2.4, which was within the values seen from other IM patients. The Frost report also documented 3 cases of IMs where there was detectable codeine concentrations in post-mortem blood (range 480–2990 µg/L) with an absence of morphine, M3G and M6G.¹⁰ These observations support the hypothesis that reduced 2D6 metabolism is associated with low or absent morphine levels.

Table 2 compares the ratio of free morphine to morphine glucuronide, which is a function of UGT 2B7 enzyme activity. This genotype was not evaluated in either the Persson⁹ or Frost¹⁰ reports, and therefore presumed to be wildtype (*1/*1). The patient in this case report had no free morphine, in contrast to the patients with the other patients who were able to produce both M3G and M6G. This could be explained by the individual's UGT 2B7 genotype of EM and UM. In addition to these genotypes, the woman in the case report had also taken fluoxetine and bupropion on the day of the accident (Fig. 1).

5. Discussion

In California, an individual who drives a motor vehicle and has a blood or breath alcohol content of $\geq 0.08\%$ can be charged with violation of Vehicle Code 23152(b), "driving under the influence" (DUI) irrespective of whether or not the individual is actually impaired. In the case where there is no alcohol involved, or if the impairment is due to the presence of illicit, prescription, and over-the-counter drugs, the individual can be charged for DUI under Vehicle Code 23152(a). There is no blood concentration for any drug that defines impairment in California, therefore the application of the law is subjectively based:

"Physical or mental abilities are impaired to such a degree that the individual no longer have the ability to drive with the

caution characteristic of a sober person of ordinary prudence under the same or similar circumstances".

The defendant in this case was tried and convicted on the basis of the blood codeine concentration, and its presumed physiologic effects. The toxicology report on the result from the defendant's blood sample included reference ranges for all positive findings to include blood codeine levels which were cited as "effective level: 0.01–0.10 mg/L and potentially toxic: 0.2–5.0 mg/L."¹¹ The free codeine result of 348 µg/L seen in this case exceeded the 300 µg/L cutoff established by The International Association of Forensic Toxicology¹² as consistent with toxicity. It is also consistent with mean serum codeine concentration of 348 µg/L observed in 9 drivers suspected of impairment from England and Wales.¹³

It is problematic to assume intoxication or impairment from opiates or opioids based solely on whether the blood level was within or exceeded a reference range value, particularly with a drug, such as codeine, that requires bioactivation to an active metabolite. Assessment of impairment from an opiate or opioid must be based on chronicity of use to ascertain tolerance. Systematic literature reviews of opiate effects on impairment of driving have concluded that there is strong evidence that there is no greater incidence in motor vehicle violations/motor vehicle accidents versus comparable controls of opioid-maintained patients, as well as consistent evidence of no impairment as measured in driving simulations of opioid-maintained patients.¹⁴ The risk is more likely to occur with the first few days of therapy in opioid-naïve patients.¹⁵ The defendant had been prescribed codeine medications consistently for years prior to the accident and most probably had achieved some level of tolerance.

Codeine, as a substrate for CYP 2D6, is susceptible to drug interactions from inhibitors of CYP 2D6. Studies using human

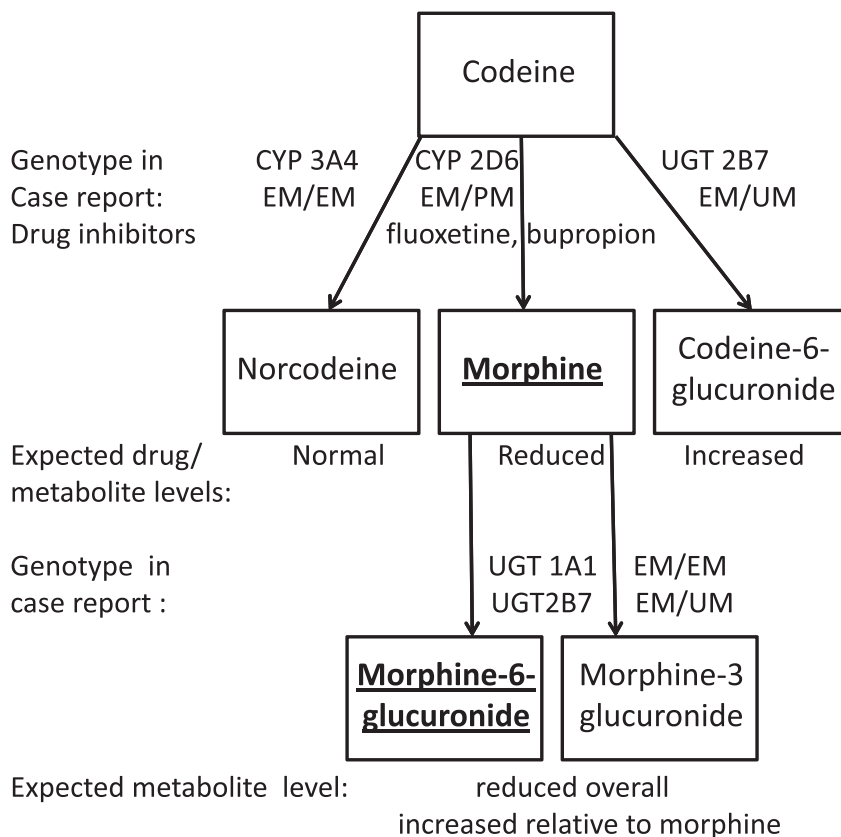


Fig. 1. The metabolism of codeine to norcodeine via CYP 3A4, morphine via CYP 2D6, and codeine-6-glucuronide via UDP 2B7. Morphine is further metabolized to morphine-6-glucuronide and morphine-3-glucuronide via UDP 2A1 and 2B7. The specific genotypes for these metabolizing enzymes for the defendant is shown.

volunteers have reported that administration of strong CYP 2D6 inhibitors diminish the pharmacological effects of codeine due to inhibition of the formation of the active metabolite, morphine.^{16–19} Fluoxetine and bupropion are antidepressants that were taken by the subject. Using human liver microsomes in vitro, Otton et al. showed that fluoxetine inhibited the conversion of oxycodone to oxymorphone from the liver of extensive 2D6 metabolizers.²⁰ Romach et al. administered 20 mg/day of fluoxetine to 8 chronic codeine users and showed a decrease in CYP 2D6 activity and an increased ratio of urinary codeine to morphine.²¹ While bupropion is also a potent CYP 2D6 enzyme inhibitor,²² data directly linking bupropion inhibition to codeine metabolism is lacking. The defendant had been administered these 2 strong CYP 2D6 inhibitors while taking codeine which could also explain the lack of morphine produced as well as lack of a pharmacologic effect from codeine. The use of the promethazine with codeine cough medication was not acknowledged throughout the investigation. However, even if it was an additional source for codeine, it would be subject to the same drug interaction as any other source.

From the combination of genetic and toxicologic data, lack of a specific and sensitive physiological marker (i.e., miosis), codeine nullifying drug–drug interactions, and chronicity of use, we conclude that the individual in this case was not impaired by codeine at the time of her traffic accident. This conclusion is consistent with her physical and medical examination conducted on her at the scene of the accident by paramedics and emergency department presentation. Her genetic makeup is such that she is unable to produce significant amounts morphine from codeine and will not benefit from the analgesic effect of the drug. Moreover, what little morphine that can be produced is quickly further metabolized to its inactive metabolites, i.e., M3G. While her free and total codeine concentrations were high, the free morphine concentrations were negligible. Of the active morphine forms, there was no free morphine and the M6G was only 3 µg/L. The subject of this case report was unusual because she was both a CYP 2D6 IM and UGT 2B7 UM. The defendant's attorney has petitioned the court for Writ of Habeas Corpus. The outcome of the petition is pending. This may be the first trial where pharmacogenomics data was used in an attempt to set aside a court ruling.

Ethical approval

None.

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Conflict of interest

Neither author declare any conflicts of interest.

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